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Microsatellite Instability in Sacral Chordoma

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Background and Objectives: Microsatellite instability (MIN) is an indirect marker of globally defective DNA mismatch repair in the neoplastic cells of cancer patients. Chordomas are rare, primary skeletal malignancies for which few characteristic molecular genetic markers have been identified. Is MIN demonstratable in chordoma?

Methods: We evaluated sacral chordomas from 12 patients with sacral chordomas for the presence of MIN at 9 different genetic loci from chromosomes 1p, 5q, 7q, 9p, 11p, 12p, 13q, 17p, and 18q. Cells were scraped from glass slides so that tumor and control DNA could be isolated and then amplified by polymerase chain reaction (PCR). Heterozygosity indices were ≥ 0.70.

Results: Six patients (50%) demonstrated MIN for at least 1 locus, and 2 patients demonstrated loss of heterozygosity (LOH) for at least 1 locus. Only 1 individual's chordoma manifested microsatellite instability (MIN) and loss of heterozygosity (LOH). Another patient manifested no MIN but LOH at 9p and 18q. Interestingly, this individual had the most aggressive clinical cancer course, presenting with lymph node metastasis and succumbing to widespread metastatic disease.

Conclusions: Chordomas can be added to the list of malignancies demonstrating MIN. LOH may prove to portend a worse prognosis than MIN when more tumors are examined.

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Key Words: sarcoma; loss of heterozygosity; PCR; chordoma; microsatellite instability

INTRODUCTION

Chordomas are rare, slow-growing, primary malignant skeletal neoplasms. Half of chordomas arise in the sacrococcygeal region. They represent < 1% of skeletal sarcomas, yielding an annual USA incidence of approximately 25 afflicted persons [1].

Chordomas arise in the remnant notochord. The notochord reaches maturity in the 11 mm embryo. In the second gestational month, the notochord obliterates. Its tissue rests are displaced from central to cranial and caudal. They leave behind microscopic foci of notochord tissue in the most cranial and caudad of vertebral bodies. Malignant transformation typically presents in the third/fourth decades for spheno-occipital lesions and in the fifth/sixth decades for sacrococcygeal chordomas (Fig. 1).

Cytogenetic analysis has been performed on 18 chordomas. No specific or characteristic chromosomal anomaly has been determined. Many cases have been hypodiploid or near diploid. Numerical or structural alterations in chromosomes 3 and 21 have been observed [2].

The chromosomal telomere in chordoma has been investigated. Telomere elongation was identified in 4 of 4 chordomas [3]. In marked contrast, telomere length reduction has been observed during the in vitro senescence

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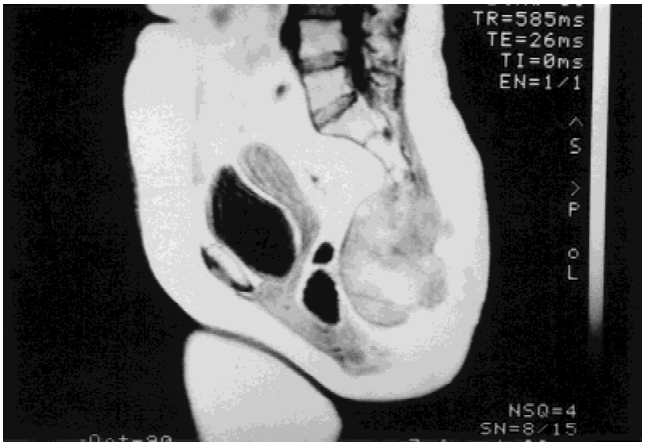

Fig. 1. Midline sagittal T1-weighted magnetic resonance image of an S3 chordoma. The tumor has a large presacral extraosseous soft-tissue mass that extends to the coccyx and S2.

of human fibroblasts and most cancers. Telomerase is the enzyme required to maintain telomere integrity. Telomerase activity was present in half of studied chordoma patients [3].

The retinoblastoma (Rb) gene is a well-characterized tumor-suppressor gene located at 13q14. It is composed of 27 exons, 26 introns, and codes for a protein 928 amino acids long. The protein binds to nuclear DNA and plays a key role in cell-cycle regulation. Its inactivation has been associated with a number of malignancies including osteosarcoma, bladder carcinoma, and breast cancer. Forty percent of high-grade osteosarcomas have an Rb deletion [4]. Two of 7 chordomas demonstrated loss of heterozygosity (LOH) at intron 17 [5].

Microsatellites are short oligonucleotide repeats dispersed throughout the human genome, which exhibit length polymorphism. Some neoplastic cells show changes in the length of their microsatellites compared with normal cells, indicating that DNA sequences have gained or lost base pairs during tumor replication. Allelic size alteration, termed microsatellite instability (MIN), are valuable genetic markers for the altered phenotypes seen in many cancers. MIN is a signal of defective DNA mismatch repair. It has been reported in sporadic carcinomas (11%–34%) and in sarcomas (44%) [6,7].

Chordoma exhibits several types of genomic anomalies. Its clinical course is protracted and varied. The purpose of this study is to determine whether MIN exists in chordoma and whether it can be correlated to outcome.

MATERIALS AND METHODS

Archival paraffin-embedded tumor blocks were retrieved after confirming the pathologic diagnosis. Ten-micron thick unstained slides were prepared from each tumor-rich paraffin block with a single hematoxylin-eosin-stained lead slide. Tumor and normal tissue were carefully scraped from the slides with disposable scalpels. The tissue was then deparaffinized and DNA isolated.

The 12 patients from whom chordoma was obtained had their clinical features catalogued. The date of definitive sacral chordoma surgery was subtracted from the most recent clinical and radiological exam to calculate "months followed." The age, gender, and whether or not radiation was administered was recorded. Negative tumor margins imply no microscopic residual tumor was left at the surgical site based on the pathologic review of the resected specimen. Disease status was recorded at the most recent clinical follow-up examination.

Forward polymerase chain reaction (PCR) primers were end-labeled with gamma^{32}P-dATP (Amersham, Arlington Heights, IL). PCR amplification was performed following established protocols at 9 loci: 1p22 (D1S187), 5q11.2–13.3 (D5S107), 7q (D7S594), 9p21 (IFNA), 11p15.2 (D11S861), 12p13.2–13.3 (vWF-TNR), 13q14.2 (D13S170), 17p13.1 (D17S786), and 18q11 (D18S34). Heterozygosity indices for the primers were ≥ 0.70 [8]. Oligonucleotide primers were obtained from Research Genetics (Huntsville, AL). Approximately 40 ng of neoplastic and control DNA was used for each primer.

PCR conditions varied according to the primer selected. Primers D18S34, D5S107, D1S187, and IFNA underwent 27 step cycles with 30 sec at 94°C, 75 sec at 55°C, 15 sec at 72°C, and then 6 min at 72°C. Primer D17S786 underwent 27 step cycles with 1 min at 94°C, 2 min at 55°C, 2 min at 72°C, and then 10 min at 72°C. Primer D11S861 underwent 25 step cycles with 1 min at 94°C, 2 min at 57°C, 2 min 30 sec at 72°C, and then 10 min at 72°C.

Following PCR, the samples were denatured at 95°C for 6 min. The samples were then immediately placed on ice, and 3.0 μl of each sample was loaded onto a 6% acrylamide gel. Electrophoresis was performed at 90 W for 90 min and, after cooling, the gel was transferred to gel blot paper and dried. The gel blot paper underwent autoradiography with exposure for 1 hr to 96 hr at −70°C prior to X-ray film developing and examination.

MIN was recorded when an insertion/deletion of repeat units (replication error) was observed in at least 1 tumor DNA locus compared with its non-neoplastic control. LOH was observed when the radiographic signal of 1 allele was reduced in tumor DNA compared with control in those patients heterozygous for a specific locus on repeated analyses.

RESULTS

The clinical and genetic instability results for 12 patients at 9 loci are displayed in Table I. Amplification of every tumor with each probe was not uniformly success-

TABLE I. Clinical and Genetic Data for Sacral Chordoma Patients

Patient no.	Age/sex	Margin of tumor resection	Radiation	Months followed	Status*	MIN	LOH
1	47/F	Negative	No	46	NED	17p	
2	37/F	Positive	Yes	120	NED	7q,17p	17p
3	48/M	Negative	No	116	NED	7q	
4	56/F	Negative	No	84	Dead		
5	68/M	Positive	Yes	96	NED	13q	
6	66/M	Negative	Yes	34	NED		
7	71/F	Positive	No	15	DOD	7q,13q	
8	47/F	Positive	Yes	13	NED		
9	59/F	Positive	Yes	32	NED	13q,18q	
10	58/M	Negative	No	70	NED		
11	34/F	Positive	Yes	63	DOD		9p,18q
12	70/F	Positive	Yes	12	NED		

*Status: NED = No evidence of disease; DOD = Dead of disease; Dead = No autopsy.
MIN = microsatellite instability (replication error at locus indicated).
LOH = loss of heterozygosity (at indicated locus).

ful. Even after repeat analysis, DNA probed with IFNA was not interpretable in 5 of 12 cases. While this probe was least reliable, D1S187 and D5S107 produced 100% interpretable results for every chordoma. In total, for the 12 × 9 grid, 22 of 108 samples were not successful (80% successful). IFNA yielded 5 unsuccessful samples: D18S34; vWF-TNR; D11S861 = 4; D7S594, D13S170 = 2; and D17S786 = 1.

Altered mobility of alleles, indicative of MIN, was seen in 6 of 12 chordomas. LOH was observed in 2 patients. One patient (no. 2) had MIN and LOH, and 1 patient (no. 11) had LOH at 2 loci without MIN. Figure 2 demonstrates microsatellite data generated by PCR amplification of genomic DNA from normal and tumor tissue. No LOH was demonstrated at 13q (Rb).

DISCUSSION

Chordomas are of 3 overlapping and sometimes coexisting histopathologic types: conventional, chondroid, and malignant spindle subcomponents. All material analyzed in this study represented the conventional and most common histopathology. No high-grade malignant spindle cell components were identified in any chordoma specimens.

Physaliferous cells are the hallmark of chordomas. Mi-

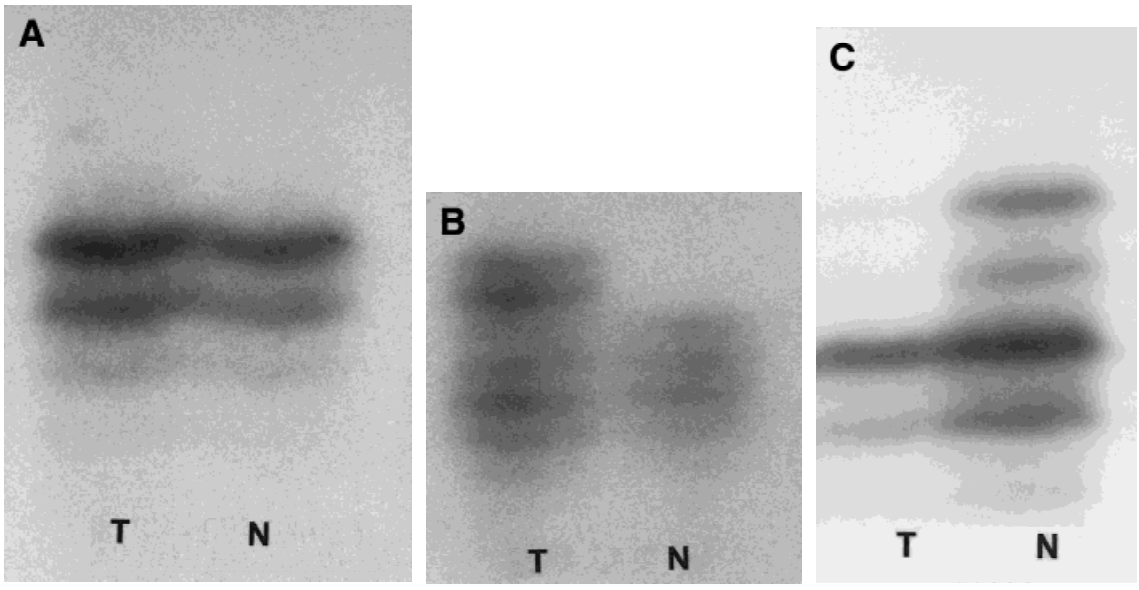

Fig. 2. Microsatellite analysis in chordoma, tumor (T) vs. normal (N). **(A)** Patient 6: probe 5q (D5S107) demonstrating no altered mobility of alleles; **(B)** Patient 7: probe 7q (D7S594) demonstrating microsatellite instability (MIN) via tumor replication error; **(C)** Patient 11: probe 18q (D18S34) demonstrating loss of heterozygosity (LOH) in tumor.

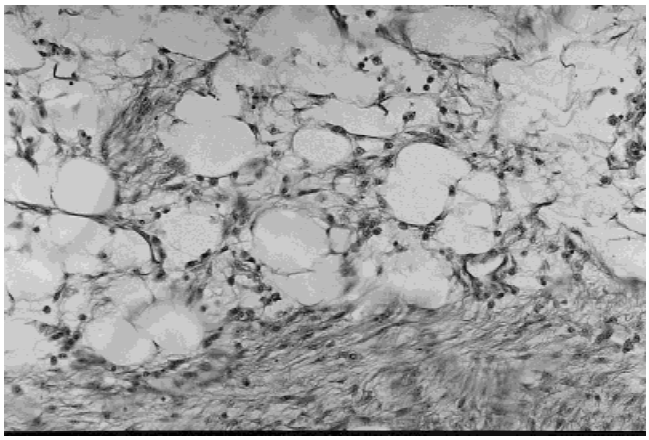

Fig. 3. Photomicrograph of a hematoxylin-eosin stain (125×). Physaliferous cells are demonstrated in a mucoid matrix.

croscopically, these polyhedral cells have distinct cytoplasmic membranes with intracytoplasmic vacuoles. The mucin produced by the cells also tends to collect outside the cells creating large nests of bubbly cells in a mucoid matrix (Fig. 3). Other cells with deep pink cytoplasm rest in chords anastomosing in the mucoid matrix. The nuclei remain small and darkly stained, indicative of its low-grade, minimally aggressive biologic behavior. Chordomas are immunoreactive for cytokeratin, epithelial membrane antigen, S-100 protein, vimentin, and neurofilaments. These characteristics help distinguish it from other mucin-producing bone cancers but are not prognostic.

Sacral chordoma grows slowly with vague nonspecific symptoms occurring late in its course. Typically at presentation a large presacral mass grows into and out from the sacrum. Nerve root function is compromised, producing urinary and bowel incontinence. Metastasis occurs late in approximately 10% [9]. Sacral amputation, while a supreme technical challenge, can be curative if a wide tumor-free margin is obtained. The more caudal the tumor's epicenter, the easier the surgical extirpation. Neurologic deficit results with or without surgery frequently requiring urinary and/or fecal diversions [10,11].

Despite its slow growth, chordoma is fatal without surgical treatment. The surgery is associated with significant morbidity. Basic science investigations and conventional histopathologic and immunohistochemical analysis have thus far failed to discover a unique or opportunistic biologic marker able to advance the care and management of this cancer.

CONCLUSIONS

Only 2 studies have examined the role of molecular markers in chordoma in relationship to clinical parameters [3,5]. There still remains a great deficiency in our understanding of the molecular biology of this cancer. We examined MIN in 12 chordomas and identified it in half. Two individuals demonstrated LOH, one with concomitant MIN, the other without. Clinically, the most aggressively behaving malignancy was individual no. 11. She presented with lymph node metastases (extremely rare in chordoma) and succumbed to widespread disease 5 years later. Interestingly, her tumor had LOH at 2 loci (the only sample to do so) and no MIN was detected. This suggests that microsatellite analysis may have a prognostic role in the future if larger studies confirm this preliminary finding.

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